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CASTE AND SEX DIFFERENCES IN COLD-HARDINESS IN THE SOCIAL WASPS, *POLISTES ANNULARIS* AND *P. EXCLAMANS* (HYMENOPTERA : VESPIDAE)

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SUMMARY

Elevated fructose, glucose and trehalose levels were found in *Polistes exclamans* and *P. annularis* after exposure to cold. Glycerol was found only after extensive exposure to cold or in mid-winter. In *P. exclamans* mean supercooling points (SCP) decreased to a low of — 12.9° C in February. In *P. annularis* SCP were never below — 6.8° C in the field, but laboratory acclimation at + 5° C resulted in SCP of — 10.7° C. Freezing was lethal to adult *Polistes* under all circumstances.

Workers and future queens were separated on the basis of the appearance of their fat bodies. For *P. exclamans*, only 17 % of the future queens died during 15 days at + 5° C (N = 29) while 76 % of the workers died during identical cold exposure (N = 45). The surviving future queens had elevated cryoprotectant levels consisting mainly of fructose in their hemolymph, while the few workers that survived did not. These experiments indicate that there are physiological differences between workers and future queens in *Polistes* collected in autumn and that fat body appearance determined using non-invasive techniques is a reliable indicator of caste. Males were similar to future queens in their response to cold indicating that autumn mating activity may extend into winter.

RESUME

La résistance au froid en fonction de la caste et du sexe chez les guêpes sociales Polistes annularis et P. exclamans (Hymenoptera : Vespidae)

Des taux élevés de fructose, de glucose et de tréhalose ont été mesurés chez *Polistes exclamans* et *P. annularis* après traitement par le froid. Par contre la présence de glycérol n'a été détectée qu'après un traitement prolongé au froid ou pendant le milieu de l'hiver. En février, le point de surgélation chez *P. exclamans* était descendu à — 12,9° C, tandis que chez *P. annularis* une température de — 10,7° C a été relevée après acclimatation au laboratoire à une température de 5° C et jamais moins de — 6,8° C dans l'habitat naturel. En toutes circonstances, la congélation s'est révélée léthale chez les *Polistes* adultes.

Les ouvrières et les fondatrices-filles furent séparées en se basant sur l'aspect de leur corps graisseux. Chez *P. exclamans*, seulement 17 % (N = 29) des fondatrices-filles et 76 % (N = 45) des ouvrières moururent après une incubation à + 5° C pendant 15 jours. Les fondatrices-filles survivantes avaient, dans leur hémolymphe, un taux élevé de fructose, qui joue donc un rôle antigel. Ces expériences démontrent qu'il existe une différence entre les ouvrières et les fondatrices-filles récoltées en automne et que l'aspect de leur corps graisseux vu à travers sa membrane est un indice de caste sûr. La réaction au froid des mâles était la même que celle des fondatrices-filles, indiquant ainsi que leur activité reproductrice de l'automne peut se prolonger en hiver.

INTRODUCTION

Primitively eusocial insects have been the focus of much research on the evolution of group living (West Eberhard, 1975 ; Wilson, 1975 ; Alexander, 1974 ; Jeanne, 1980 ; Strassmann, 1981 ; Strassmann and Meyer, 1983). Workers and queens of *Polistes* are morphologically identical, and under many conditions workers may become queens (Litte, 1979 ; Richards, 1978 ; Strassmann and Meyer, 1983). One of the key ways in which workers differ from foundresses is that foundresses overwinter while workers do not. Therefore caste differences are most likely to be found in ability to survive cold weather.

Females found on autumn nests can be divided into two classes : (1) those who are inactive and will soon hibernate, subsequently becoming the next year's nest foundresses and (2) those who are foraging workers, and are considered incapable of hibernating (West Eberhard, 1969 ; Eickwort, 1969 ; Noonan, 1981). The behavioral differences between these two types of females have been widely noted (West Eberhard, 1969 ; Noonan, 1981 ; Rau, 1928, 1929 ; Strassmann (1979). Eickwort (1969) found that workers differed from foundresses in autumn in the quantity and consistency of their " fat ". Foundresses contain " a thick continuous blanket of fat, approximately 0.5 mm thick or thicker. This fat, when fixed in Dietrich's solution, had the general appearance of tiny clear spheres embedded in a milky-white matrix (Eickwort, 1969, p. 68) ". Eickwort (1969) noted that in workers : " the parietal fat body was discontinuous, segmentally arranged, thin and patchy, and appeared more opaque, yellowish, and lumpy ". In a study of *Mischocyttarus flavitarsis*. Litte (1979) found marked females who were known to have foraged in hibernacula in the middle of winter. Therefore it is possible that there are no real physiological differences between autumn workers and foundresses and that if a worker lived to the end of the season she could mate and hibernate and become a foundress the following spring, just as a mid-summer worker can mate and immediately become an egg-laying queen. In temperate regions adult females overwinter in hibernacula that are somewhat protected from temperature extremes (BAUST, 1976; GIBO, 1972). These hibernacula include cracks in cliffs, crevices under tree bark, attics, old nests of *Dolichovespula* (Hymenoptera : Vespidae), sheds and tree stumps (RAU, 1930; BOHART, 1942; SNELLING, 1954; GIBO, 1972, 1976, 1980; HERMANN *et al.*, 1974; STRASSMANN, 1979). *Polistes* are often found hibernating in groups which include females from several nests of different species (BOHART, 1942; HERMANN *et al.*, 1974; SNELLING, 1954). Males survive into autumn and for part of the winter since mating takes place in late autumn (*P. exclamans*) or midwinter (*P. annularis*) (RAU, 1928, 1930 : STRASSMANN, 1979, unpubl.).

The purpose of this study was to determine whether or not differences exist in ability to acclimate to cold between autumn workers and foundresses. Another goal was to determine whether or not the appearance of the fat body was a good indicator of caste (worker or foundress). To answer these questions it was first necessary to describe in general the phenomenon of cold acclimation in *Polistes*. This was done by analyzing cryoprotectant levels and supercooling points in wild-caught wasps throughout the year and by subjecting pre-hibernation foundresses and summer workers to 3 levels of cold for varying intervals and then determining how this affected their supercooling points and hemolymph cryoprotectant levels.

MATERIALS AND METHODS

P. exclamans was collected in Austin (30.2° N, 97.5° W) and Houston (29.5° N, 95.2° W), Texas according to methods outlined by STRASSMANN (1979). *P. annularis* was collected from a study site about 30 miles west of Austin, Texas. *P. instabilis* was collected in McAllen, Texas (26.2° N, 98.2° W). Field samples of wasps were analyzed within 3 hours to 3 days. Before analysis wasps were maintained at the temperature at which they had been collected and were not fed.

To examine cold acclimation in foundresses, individuals were subjected to constant temperatures of + 5° C, + 10° C or + 15° C in low temperature incubators at 100 % humidity in complete darkness. Each container held a group of at least 5 wasps since they hibernate in groups naturally. Wasps were not fed during acclimation. *P. exclamans* were removed from the cold after 7, 15 and 23 days, and their supercooling points, and hemolymph carbohydrates were measured. *P. annularis* were removed after 9 and 16 days. *P. exclamans* collected February 26 were acclimated 7 days at 0° C.

Caste differences in ability to cold acclimate were studied by subjecting males, foundresses and workers to + 5° C for varying amounts of time. This temperature was chosen because the above experiments indicated that it resulted in the greatest increase in cryoprotectant levels.

Workers were separated from foundresses in *P. exclamans* and *P. annularis* in late September by careful inspection of their fat bodies. Individuals were cooled to + 8° C so that they could be handled easily. Wasps were positioned ventral side up under a Wild dissecting microscope at 20 x. Fat was examined by gently pulling on the tip of the abdomen with forceps until the fat became visible through the clear intersegmental membrane between the hard cuticle on each segment. In workers this fat appeared sparse, stringy, and yellow. In foundresses two forms of fat occurred. Most common was a thick layer of solid white fat that was somewhat segmented and readily

Table I. — Seasonal values (X ± SEM) for carbohydrate levels (µg/mg) (1) and supercooling points (SCP) for field-collected *Polistes exclamans* and *P. annularis.*

Tableau I. — Valeurs saisonnières (X ± SEM) des taux d'hydrates de carbone (µg/mg) et points de surgélation chez *Polistes exclamans* et *P. annularis* récoltées dans leur habitat naturel.

Date	Fructose	Glucose	Trehalose	Glycerol	SCP (°C)
P. exclamans					
Foundresses					
Sept. 14	3.6 ± 0.5	7.9 ± 0.5	7.1 ± 0.4	0	— 8.2 ± 0.7
Oct. 15	6.5 ± 1.2	10.9 ± 1.5	9.1 ± 1.1	0	— 7.9 ± 1.1
Oct. 20	2.4 ± 0.1	4.3 ± 0.6	6.7 ± 0.5	0	— 10.6 ± 0.9
Oct. 24	6.6 ± 1.8	11.1 ± 1.5	4.7 ± 1.1	0	— 9.9 ± 0.9
Feb. 8	3.2 ± 2.1	5.1 ± 2.1	10.5 ± 0.0	2.6 ± 1.6	— 12.9 ± 1.3
Feb. 26	1.7 ± 0.4	4.4 ± 1.3	11.6 ± 1.6	1.3 ± 0.7	— 6.7 ± 0.4
May 12	2.8 ± 0.1	2.7 ± 0.7	5.1 ± 0.8	0.8 ± 0.1	— 9.3 ± 0.5
May 26	0.8 ± 0.4	1.7 ± 0.3	2.6 ± 0.9	0	— 6.3 ± 1.1
Workers					
Aug. 11	2.3 ± 0.1	3.0 ± 0.2	0.7 ± 0.0	0	— 5.3 ± 0.1
May 12	5.1 ± 0.6	6.0 ± 0.2	4.1 ± 0.0	0.2 ± 0.0	— 9.9 ± 1.1
May 26	0	1.3 ± 0.8	0	0.4 ± 0.1	— 5.9 ± 0.2
June 8	6.3 ± 1.6	6.8 ± 0.4	3.5 ± 0.8	1.1 ± 0.1	
Males					
Oct. 20	18.5 ± 1.3	24.4 ± 1.8	8.5 ± 0.5	0	— 9.0 ± 0.9
Oct. 24	12.5 ± 3.3	22.0 ± 3.1	6.5 ± 0.6	0	— 10.3 ± 0.9
P. annularis					
Foundresses					
Nov. 2	1.0 ± 0.1	4.7 ± 1.0	6.5 ± 0.4	0	— 6.3 ± 0.5
Feb. 26	1.9 ± 0.2	3.4 ± 0.2	10.4 ± 1.5	0.2 ± 0.1	— 6.8 ± 0.3
May 26	2.0 ± 0.6	1.5 ± 0.6	4.8 ± 0.5	0	— 6.7 ± 0.4
Workers					
May 26	2.6 ± 0.5	2.9 ± 0.6	3.2 ± 0.4	0.6 ± 0.2	— 6.8 ± 0.1

(1) µg/mg wet weight.

A single freezing event, initiated in either body region, was lethal for either species regardless of the season of field collection or the extent of low temperature acclimation in the laboratory. Even at rates as low as 0.3°C/min., the wasps did not survive tissue freezing. Therefore the first supercooling event for an individual represents the lower lethal temperature, since wasps survive exposure to temperatures immediately above their SCP. Mean values given *in table I* were obtained by averaging the higher supercooling point for each 5-10 individuals.

Experimental laboratory acclimation

After 16 days at + 5°C females of *P. annularis* elevated their trehalose levels four-fold, and glucose two-fold, over initial values *(fig. 1)*. Wasps maintained at + 15°C had the lowest carbohydrate levels. A similar but less pronounced response was observed for *P. exclamans*. Females acclimated at + 5° C exhibited higher concentrations of trehalose, glucose and fructose than ones held at warmer temperatures *(fig. 2)*. Male *P. exclamans* also elevated trehalose levels at + 5°C, while other treatments maintained or decreased cryoprotectant concentrations.

When female *P. exclamans* collected in February were cold acclimated for one week at 0°C, their SCP went from — 12.0°C up to — 4.8°C (N = 5) and — 6.1°C (N = 3, Dying individuals) and glycerol concentrations in their hemolymph increased from 0 to 0.25 and 3.2 μg/mg.

Caste differences in ability to acclimate to cold

By 25 September when wasps were collected for caste experiments there were very few worker *P. annularis* available to sample. Half of the workers were dead after 15 days at + 5°C, while none of the foundresses had died at this point *(table II)*. Fifteen day survivorship was significantly greater in foundresses than workers ($\chi^2 = 17.8$, $p < 0.001$, N = 68). Workers did not differ from foundresses in cryoprotectant concentrations, but the sample was very small *(table III)*. Eight percent of males were dead after 15 days and 25 % were dead after 33 days *(table II)*. Male mortality after 15 days did not

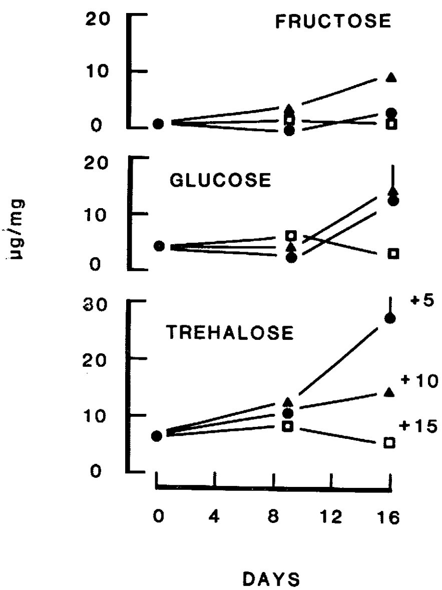

Fig. 1. — Accumulation of fructose, glucose and trehalose in females of *P. annularis* during 16 days of cold exposure at + 5° C, + 10° C and + 15° C, under constant dark conditions.

Fig. 1. — Accumulation du taux de fructose de glucose et de tréhalose chez les femelles de *P. annularis* pendant une incubation de 16 jours à + 5° C, + 10° C et + 15° C, et dans une obscurité constante.

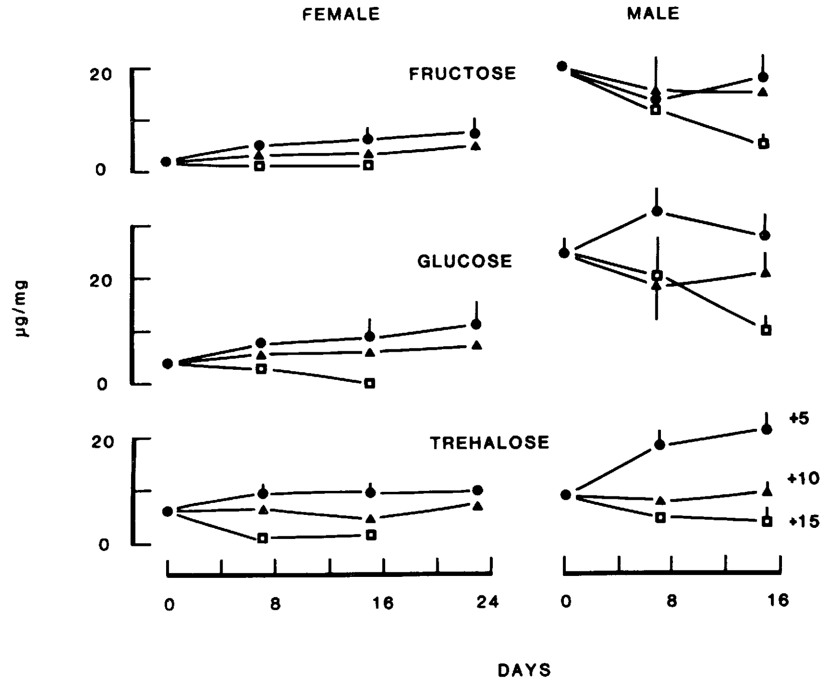

Fig. 2. — Accumulation of fructose, glucose and trehalose in females and males of *P. exclamans* during 16 and 24 days of cold exposure at + 5° C, + 10° C and + 15° C, under constant dark conditions.

Fig. 2. — Accumulation du taux de fructose, de glucose et de tréhalose chez les femelles et chez les mâles de *P. exclamans* pendant une incubation de 16 à 24 jours à + 5° C, + 10° C et + 15° C, et dans une obscurité constante.

differ from that of foundresses ($\chi^2 = 1.92$, $p > 0.05$, $N = 92$). Males had levels of glucose, fructose and trehalose that were higher than those of foundresses *(table III)*.

Seventy-six percent of worker *P. exclamans* collected in September died after 15 days at + 5° C while only 17 % of the foundresses died ($\chi^2 = 41.7$, $p < 0.001$, $N = 74$). Those workers that lived did not accumulate cryoprotectants in their hemolymph *(table III)*. Foundresses had elevated levels of fructose. Male response to cold was similar to that of foundresses. There were no significant differences between male and foundress survival after 15 days at + 5°C ($\chi^2 = 0.01$, $p > 0.05$, $N = 58$). Only 20 % died after 15 days at + 5°C, and they had elevated levels of both fructose and glucose *(table III)*.

None of the workers collected in June increased cryoprotectant levels. After 13 days at + 5°C 70 % of *P. annularis*, 72 % of *P. exclamans* and 97 % of *P. instabilis* were dead *(table II)*. Mid summer workers thus appear to be incapable of acclimating to cold.

Table II. — Survivorship of foundresses, workers and males after cold acclimation at + 5° C.

Tableau II. — Survie des fondatrices-filles, ouvrières et mâles après acclimatation à une température de + 5° C.

A. Wasps collected 25 September 1982

	Days at 5° C	Foundresses % dead	Foundresses N	Workers % dead	Workers N	Males % dead	Males N
Polistes annularis	15	0	64	50	4	8	24
	33	20	40	0	2	25	16
Polistes exclamans	15	17	29	76	45	20	5
	33	100	24				

B. Wasps collected 8 June 1982

	Days at 5° C	Workers % dead	Workers N
Polistes annularis	1	0	31
	8	7	27
	13	70	23
Polistes exclamans	1	0	73
	8	34	65
	13	72	57
Polistes instabilis	1	0	68
	8	22	60
	13	97	60

DISCUSSION

Gibo (1972, 1976, 1980) found that foundresses of *P. fuscatus* were able to survive mid-winter temperatures of — 20°C in Ontario, Canada in minimally protected shelters. In all cases they were freezing intolerant (Gibo, 1976).

Vespula maculata are tolerant of ice formation in their hemolymph (Duman and Patterson, 1978). Winter SCP were only — 4.6°C, but adults were able to survive temperatures as low as — 14°C by tolerating ice formation in their hemolymph (Duman and Patterson, 1978).

Supercooling points of *P. exclamans* and *P. annularis* range between — 10°C and — 13°C at their lowest, indicating they would not be able to survive Ontario winters. However these SCP are well below minimum temperatures in central Texas. Unlike *V. maculata*, *P. exclamans* and *P. annularis* are frost intolerant, though they can withstand similar temperatures. Further work with mid-winter wasps acclimated at 0°C is necessary to determine whether or not the rising SCP we observed with February wasps is a general phenomenon indicating the presence of nucleators.

Foundresses of *P. exclamans* are able to cold-acclimate by elevating

Table III. — Responses of foundresses, workers and males to cold acclimation at + 5° C.

Tableau III. — Réaction des fondatrices-filles, ouvrières et mâles après acclimatation à une température de + 5° C.

A. *Polistes annularis*

Days at + 5° C		Foundresses		Workers		Males	
		X ± S.E.	N	X ± S.E.	N	X ± S.E.	N
Super-cooling	4					— 8.7 ± 0.6	10
point (1)	15	— 9.5 ± 0.6	10	— 7.4 ± 1.2	4	— 9.1 ± 0.8	6
	33	— 8.7 ± 1.6	11	— 10.7 ± 0.2	2	10.6 ± 0.5	10
Glucose	4					1.0 ± 1.0	3
(µg/mg)	15	1.5 ± 0.3	3			1.5 ± 0.7	3
	33	0.7 ± 0.2	3	2.1 ± 0.6	2	4.4 ± 1.6	3
Fructose	4					2.1 ± 0.6	3
(µg/mg)	15	1.9 ± 1.4	3			1.0 ± 0.2	3
	33	2.5 ± 0.6	3	14.4 ± 1.0	2	14.2 ± 7.0	3
Trehalose	4					1.1 ± 1.1	3
(µg/mg)	15	2.2 ± 1.2	3			4.0 ± 0.7	3
	33	2.1 ± 1.2	3	9.0 ± 2.6	2	4.2 ± 2.0	3

B. *Polistes exclamans*

		Foundresses X ± S.E.	N	Workers X ± S.E.	N	Males X ± S.E.	N
Super-cooling	2	— 10.8 ± 0.8	12	— 10.4 ± 0.9	10	— 10.8 ± 0.5	5
point	15	— 9.9 ± 0.6	17	— 10.0 ± 0.4	10	— 12.1 ± 1.0	2
Glucose	2	3.3 ± 0.6	3	3.2 ± 0.4	3	3.4 ± 0.7	3
(µg/mg)	15	1.9 ± 0.9	2	2.9 ± 0.5	3	6.3	1
Fructose	2	1.6 ± 0.2	3	1.7 ± 0.3	3	2.9 ± 0.9	3
(µg/mg)	15	8.8 ± 0.4	2	2.7 ± 0.7	3	13.0	1
Trehalose	2	1.1 ± 1.1	3	2.4 ± 0.9	3	2.6 ± 0.9	3
(µg/mg)	15	3.8 ± 1.5	2	4.2 ± 0.9	3	1.8	1

(1) Since the SCP of abdomen and thorax differed in each individual and since neither consistently nucleated first, and the first SCP was always lethal, the first SCP was used to calculate means.

cryoprotectant levels at + 5°C and have high survival rates when maintained at this low temperature. The workers were not able to elevate their cryoprotectant levels at + 5°C and had low survivorship when maintained at this temperature. Both the cold-acclimated workers and foundresses had similar supercooling points. The elevated cryoprotectant levels in the foundresses may be providing protection at the low temperature (+ 5°C) by maintaining membrane fluidity, stabilizing protein structures and reducing transmembrane fluxes.

The difference noted in the type of fat stores between foundresses and workers may also be reflective of the ability of the one caste to cold-harden and the other's inability to cold-harden. If the foundresses have higher glycogen stores than the workers, these may be mobilized at low temperatures to enhance cryoprotectant levels.

Workers can be separated from foundresses on the basis of the appearance of their fat body as viewed through the intersegmental membrane. The difference in cold tolerance and cryoprotectant accumulation between the two castes is great in *P. exclamans*, indicating that there are real physiological differences between workers and foundresses at this time of year. Since this work was performed on adults, it is not possible to determine at what point the difference arises, or what the trigger is. Clearly there are no females capable of overwintering in June. Further experiments are necessary to determine whether or not flexibility still exists upon adult emergence, or whether or not caste is fixed in pupae.

Since females precede males in emergence in autumn in both species considered here, there may be a conflict of interest between workers and the queen as to when females should be channeled into workers and when they should be channeled into foundresses (STRASSMANN, 1984). Since females share 3/4 of their genes with sisters and only 1/4 with brothers, a worker should prefer to raise sisters over brothers (HAMILTON, 1972). By the same logic a worker would prefer that the sister she rears become a foundress and not a worker, especially not one that rears males. If workers win this conflict, colony cycles may end earlier than necessary due to weather factors, and male broods may be aborted and fed to foundresses. That colony cycles of *P. annularis* sometimes end in July may support this hypothesis (STRASSMANN, unpubl.) More detailed information on caste determination will resolve the question.

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